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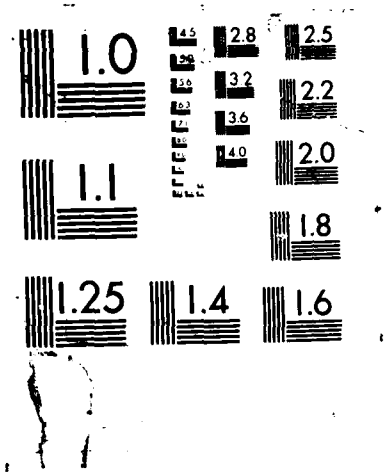
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Annual Report: Sept. 1987

Grant # N00014-85-K-0651

Principal Investigator: Marco Colombini

1. Research Accomplishments

The goal of the project is to gain a better understanding of the molecular basis underlying the voltage dependence of the mitochondrial channel-forming protein, VDAC. This year we have made progress in the following areas:

a) In the previous year we had demonstrated that low levels of modification of VDAC channels by succinic anhydride converted the channels from weakly anion to weakly cation selective while elimination their voltage dependence. Higher levels of modification made the channels much more cation selective and restored much of the voltage dependence. These results are not only consistent with earlier findings that both the selectivity filter and the sensor are composed of amino groups, but also point, for the first time, to the conclusion that the nature of the charged group on the sensor is unimportant. Charge must be present but it need not be a particular group which sits in a particular site. Indeed, it need not be a charged group of the same sign! This work is now complete and in press.

b) Further insight into the nature and location of the voltage sensor has come from studies into the effects of aqueous forms of aluminum on the properties of the channels. As little as 5 uM aluminum is solution at pH 7 will markedly reduce the voltage dependence of VDAC. Both the rate and extent of channel closure is reduced by aluminum addition. Total loss of voltage dependence occurs between 10 and 100 uM aluminum. These findings are not the result of complex non-specific interactions between aluminum and VDAC. Rather they appear to be the result of specific interaction between aluminum and VDAC's voltage sensor. The parameter, n, which is a measure of the steepness of the voltage dependence and the minimal number of charges on the sensor, decreases with increasing aluminum concentration. The voltage needed to close half the channels increases in an antiparallel fashion. The energy difference between the open and closed states in the absence of an applied voltage changes very little at the aluminum concentrations tested. Thus aluminum seems to neutralize the voltage sensor and therefore eliminates the voltage dependence. It does this without any effect on the selectivity of the channels. Thus it seems unlikely that the sensor is located within the pore of the channel. This also provides strong evidence against the hypothesis that the sensor and selectivity filter are one and the same. Finally, these results indicate that VDAC channels may have two sets of charges each operating as a sensor, one operating at positive potentials and the other at negative potentials.

c) The discovery that polyanions can increase the steepness of the voltage dependence of VDAC channels has been pursued by studying the activity of two different classes of polyanions: totally polar and amphipathic. The former consists of the molecules, dextran sulfate and polyaspartic acid, while for the latter a single molecule was used, a copolymer of styrene, methacrylate and maleate produced by Tamas Konig. Both classes

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Colombini, Marco

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16. SUPPLEMENTARY NOTATION

17. COSATI CODES			18 SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
FIELD	GROUP	SUB-GROUP	membrane channel; voltage dependence; aluminum; polyanion protein modification, mitochondrion, outer membrane, VDAC
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19. ABSTRACT (Continue on reverse if necessary and identify by block number)

The molecular mechanism underlying the voltage dependence of the mitochondrial channel, VDAC, is the general aim of the research. The approaches used to move toward that goal were: succinic anhydride modification, regulation by aqueous aluminum species and polyanions, and kinetic analysis. The results indicate that the voltage sensor and selectivity filter are distinct groups of charges, probably amino groups. The sensor is probably not located in the channel proper but on one or the other surfaces of the membrane where it can interact with high molecular weight polyanions. The kinetic studies are in their early stages but indicate the presence of unusual physical phenomena..

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dramatically increase VDAC's voltage dependence but Konig's polyanion works at lower concentrations and can cause channel closure in the absence of an applied potential. A theory has been developed to explain these observations. Using this theory and these polyanions we may be able to ask questions about the location and dynamics of the sensor.

d) The kinetics of closure of VDAC channels has yielded rather typical and expected results showing the voltage dependence of the closing rate constant. However, the voltage-dependence of the kinetics of opening are rather surprising. The rate constant seems almost voltage independent until zero potential is reached. Once this is crossed, the rate increases dramatically. These results are reminiscent of the effect of a magnetic field on the alignment of atoms of a magnetic material. Further studies are needed to substantiate and characterize further these surprising results. These experiments are very time-consuming and it is difficult to obtain results.

2. Published Papers

Mangan, P. S. and Colombini, M. 1987. Ultra-steep voltage dependence: its discovery and potential significance. Proc. Natl. Acad. Sci., U.S.A., 84:4896-4900.

Adelsberger-Mangan, D. M. and Colombini, M. 1987. The elimination and restoration of voltage dependence in the mitochondrial channel, VDAC, by graded modification with succinic anhydride. J. Membrane Biol., 98:157-168.

Dill, E.T., Holden, M.J. and Colombini, M. 1987. Voltage gating in VDAC is markedly inhibited by micromolar quantities of aluminum. J. Membrane Biol. 99: (in press)

Colombini, M. 1987. Regulation of the mitochondrial outer membrane channel, VDAC. J. Bioenerget. Biomemb., (in press)

Forte, M., Adelsberger-Mangan, D. and Colombini, M. 1987. Purification and characterization of the voltage-dependent anion channel from the outer mitochondrial membrane of yeast. J. Membrane Biol. (in press)

Colombini, M., Yeung, C.L., Tung, J. and Konig, T. 1988. The mitochondrial outer membrane channel, VDAC, is regulated by a synthetic polyanion. Biochim. Biophys. Acta (in press)

3. Published Abstracts

Dill, E.T., Holden, M.J. and Colombini, M. 1987. Physiological levels of aluminum alter the voltage-sensing mechanism of the mitochondrial channel, VDAC. The Physiologist (in press)

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